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THE CONJUNCTIVAL REACTION FOR GLANDERS.* (OPHTHALMIC TEST.)

K. F. MEYER.

(From the Laboratory of the Pennsylvania State Livestock Sanitary Board.)

During the last four years, in different parts of the world, extensive experiments have been conducted to find an accurate method by which glanders in horses can be quickly diagnosed by the practitioner without awaiting the result of one of the reliable serum tests. It has been found by Vallée,¹ Martell,² de Blicq,³ Schnürer,⁴ Müller, Gaetgens and Aoki,⁵ Fröhner,⁶ Reinhardt,⁷ Miessner,⁸ and others that the local allergic reactions may be successfully used for this purpose, and that they undoubtedly offer great advantages and greater reliability than any of the other mallein tests. By reason of the fact that mallein, compared with tuberculin, gives a more pronounced local reaction when subcutaneously applied, theoretically, better results are expected in glanders with the local tests than in tuberculosis by similar methods. The investigations of Schnürer on more than 10,000 army horses were so encouraging that it was considered advisable to experiment along similar lines in Pennsylvania.

From preliminary experiments with mallein brute of the Institut Pasteur, and with our own preparations of similar composition, it was concluded that only a specially prepared standard mallein would promise uniformly satisfactory results. The assertion of Wladimiroff⁹ that the use of an unknown, untested preparation

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¹ *Bull. de la Soc. centr. de méd. vét.*, 1907, 61, p. 359.

² *Ibid.*

³ *Ztschr. f. Infektionskrankh. usw. d. Haustiere*, 1910, 7, p. 418.

⁴ *Ztschr. f. Infektionskrankh. usw. d. Haustiere*, 1908, 4, p. 216; *ibid.*, 1912, 10, pp. 321 and 408; *Deutsche tierarztl. Wchnschr.*, 1910, 18, p. 65.

⁵ *Ztschr. f. Immunitätsf.*, 1911, 8, p. 626.

⁶ *Monatschr. f. prakt. Tierh.*, 1912, 23, p. 1; *ibid.*, p. 433.

⁷ *Monatschr. f. prakt. Tierh.*, 1911, 23, p. 178.

⁸ *Centralbl. f. Bakteriöl.*, Abt. I, O., 1912, 63, p. 482.

⁹ *Handbuch der Technik und Methodik der Immunitätsforschung*, Supplement I, 1911, p. 394; *ibid.*, 1908, 1, p. 1190.

will never permit comparative deductions, is only too true, and can without reserve be applied to many of the mallein products of commercial houses. All the ordinary "raw" mallein preparations are poor in the specific antigens and, particularly in occult cases of glanders, cause extremely weak and indistinct reactions. As none of the tested mallein preparations ("Mallein siccum Foth"; mallein of the "chemische Fabrik Humann und Teisler," etc.) used in Europe for the conjunctival tests were on the market in America, an attempt was made to prepare such a biologic product in our own laboratory. After several unsuccessful attempts, largely due to unsuitable glanders cultures, a mallein was obtained which gave unquestionably perfect results. The mallein used for this purpose is known under the name of "Mallein siccum Foth." Its preparation is given here, as it is of essential importance for the conjunctival test.

PREPARATION OF "MALLEIN SICCUM."

According to the outline given by Foth,¹ which practically has been followed in our laboratory, the preparation is as follows:

Slightly acid 2 per cent peptone and 4.5 per cent glycerin broth in wide Pasteur flasks (in the quantity of 250 c.c.) is inoculated with highly virulent glanders bacilli. The broth consists of equal parts of meat and potato extract. The glanders strain, selected for this purpose from a series of 20 strains from different outbreaks, has been passed through cats and tends to grow remarkably well on the surface of the broth. This feature is particularly important for the production of a potent mallein. For four to six weeks the cultures remain in the incubator at a temperature of 38° C. After being tested for purity and killed by heating to 70°-100° C., the fluid is concentrated slowly to $\frac{1}{10}$ of its original volume in a vacuum distilling apparatus at a temperature of 75°-80° C. The syrup-like, brownish fluid is sucked through a number of folded filters. The process frequently is extremely slow, and the loss due to the filtration is comparatively high. The clear fluid is then precipitated in absolute alcohol (1 part of mallein to 30 parts of absolute alcohol). The pouring of the fluid into the alcohol should be done carefully, as otherwise a coarse, sticky deposit will result instead of a fine, flocculent, white-brownish one. The precipitation should be done only with absolutely water-free alcohol. The precipitate is filtered off, washed with absolute alcohol or ether, and spread on dry clay plates which are afterward put into an exsiccator and, under vacuum, dried over sulfuric acid. After 24 hours, a light, whitish powder can be scraped from the clay plates; this powder dissolves very readily in water, which it tinges a dark brown. A carefully prepared powder should not be hygroscopic, and, if protected from light (one of the main characteristics of mallein toxin is that it is very labile to light), will be active for one entire year and, according

¹ *Ztschr. f. Tiermedizin*, 1911, 15, p. 401; *Ueber die Gewinnung eines festen Malleins und über seine Bedeutung usw.*, Berlin, 1896.

to the experiences of others, probably longer. According to Foth, an old powder is more elective in its antigen properties than a fresh preparation, because the unspecific pyrogenous substances disintegrate more quickly than the specific mallein substances which in powder form remain practically unaltered.

TECHNIC OF THE REACTION.

Preliminary experiments have shown that the solution of the powder disintegrates rapidly, which is due in part to the absence of preservatives and in part to changes in toxin molecules. Therefore the powder form alone can be kept in stock, and the test solution should always be made shortly before use. It was found that the best and handiest equipment for the practicing veterinarian consists of two small bottles, one containing the powder, the other, sterile or saline water in such quantity as will make a 5 per cent solution of mallein. Bottles are kept in stock with the quantities which are necessary to test 10 or 50 horses respectively. It is considered that the quantity of 0.1 c.c. solution (according to the calculations made by Schnürer) is quite sufficient to test one horse. As explained, such prepared mallein deteriorates rapidly. Therefore the solution should be used immediately after its preparation and the remainder should be discarded. Recently, with a very strong mallein, weaker solutions of the powder (1 per cent and 2 per cent) have been tested and found to be as satisfactory as when a 5 per cent solution is used.

APPLICATION OF THE MALLEIN.

The solution of mallein is instilled by means of an eye-dropper in the quantity of two to three drops; it is not necessary to work absolutely quantitatively. During the last few months, in several glanders outbreaks, the mallein has been applied to the conjunctival sac by means of a camel-hair brush, with which, naturally, quantitative working is impossible. The eye should not be handled after the instillation has been made. A successful quantitative working is impossible because the animal usually closes its eyes after successful instillation, and a certain amount of the instilled mallein is expressed with the tears. The objection that the use of a camel-hair brush may act as an agent in the distribution of glanders has been disproved by experiments of Galtier and Schnürer. Instead of the eye-dropper or brush, Müller, Gaechtgens, and Aoki have recommended the use of a glass rod. The camel-hair brush, which can be kept clean in carbolic acid when not in use, has the disadvantage of diluting the mallein solution with the conjunctival secretion. On the other hand, the application with the eye-dropper is, under ordinary circumstances, an extremely tedious task, particularly when testing young and nervous animals and when the light conditions in the stable are unfavorable. Special recommendations cannot be made and it is best to individualize according to the conditions. In any case, a careful examination of the eye in good light should be made before the mallein is applied. In one instance, a misleading result, similar to those discussed by Schnürer, was obtained in our experiments, largely due to the fact that an incipient conjunctivitis caused by a foreign body was overlooked at the time of inspection.

Schnürer frequently found an increase in temperature during the reaction and therefore advises the taking of temperatures in a manner that will be explained later. Miessner considers this rise unnecessary for a diagnosis and its detection too laborious for the benefit which can be derived from a temperature increase which is frequently only slight. Based on our own observations, the view of Miessner can, without

restriction, be supported. Only in four outbreaks previous to the application of the mallein, the temperatures were taken with "Reform" thermometers. I agree with Miessner that the temperature fluctuations are, in 98 per cent of the cases, so small that they can be recorded only when working with the greatest possible accuracy. Under the usual conditions, the results and their application for the diagnosis are, therefore, illusive and of no practical value.

REACTIONS.

In the majority of cases, shortly after the application of the mallein, slight lacrimation and perhaps an inferior degree of photophobia will be observed. For a few hours this reaction is absolutely non-specific and has nothing whatever to do with the specific reaction, which usually occurs five to seven hours after the application of the test. According to Schnürer, Fedders, and others, the specific reaction does not begin before the third hour. We did not observe any such early reaction. The specific reaction is, according to Schnürer's, Miessner's, and the writer's observations, characterized:

1. By moderate, profuse lacrimation, and pus-like, slimy secretion in the inner canthus of the eye. The degree varies considerably. Often there is only a small drop of pus; sometimes a real pyorrhea, extending in form of flakes over the entire orbital region or gumming the eyelids, and clinging to the hairs of the lids. The conjunctival sac is filled to a more or less pronounced degree with pus. The touching or the manipulation of the eyelids is in most cases extremely painful. The conjunctival membranes are deeply reddened, and in many instances there is a marked edema of the upper and lower lid, causing, therefore, a partial closure of the opening between the lids. Occasionally in such cases a glassy appearance of the mucous membranes with small petechiae is observed. The cornea and other portions of the eyeball were in none of our cases involved in the inflammatory process. Special attention was paid to these observations, as several Russian investigators (Krestowsky, Wladimiroff) claim to have observed turbidities of the aqueous humor. The inflammatory process is localized on the conjunctival membranes and is a suppurative conjunctivitis.

This reaction varies considerably in different animals and in the different stages of infection, and a scale of reactions can be noted

and should be reported as such. Only a discharge with leukocytes, that is to say, a purulent discharge, is to be considered as a positive reaction. There does not exist any uniformity of opinion as to this point of the reaction, which, in my opinion, is largely due to the fact that different mallein preparations have been used. All investigators (Schnürer, Miessner, and others), using the powdered mallein, agree on this point: *that only a discharge with pus can be considered a positive reaction.* In cases in which only a grayish, cigar-ash-like clump of a slimy discharge is found in the inner canthus, or in cases in which only a slight inflammatory reaction is noticeable, the reaction is regarded as questionable or doubtful, and marked (D); in all such cases, following the advice of Schnürer, a retest was made in 24 hours. The degree of pus in the discharge is marked P₁, P₂, P₃. To specify more clearly for the beginner, reading the ophthalmic reactions, it may be said that a conjunctivitis which can, as Schnürer states, be diagnosed at a distance should be marked P₃. With this as a standard reaction, after a few practical observations, the beginner will have no difficulties in distinguishing the other degrees. During the last month, the conjunctival test has been applied in Pennsylvania by many veterinarians who were never personally instructed as to the interpretation of the reaction, and still the results, as checked by the serum tests, were uniformly good and often recorded with remarkable accuracy.

2. Schnürer, Miessner, and others have noted, contrary to the statement of de Blieck, that in 73 per cent of the horses which gave a positive ophthalmo-reaction 12 hours after the application of the ophthalmic mallein, a temperature rise of over 38.5° C. can be recorded. Schnürer emphasizes the great importance of this febrile reaction; Miessner, on the contrary, considers its recordance as not being worth the additional labor. In our cases, in about 80 per cent of the horses tested, the rectal temperature increased from 2° to 4° F. in the 18 hours following the application of the mallein, and decreased gradually toward the 24th hour. Naturally, only afebrile cases could be tested in these experiments. This temperature reaction takes place only in animals which are actually affected with glanders. Many comparative tests on a

fairly large number of healthy horses have shown that no temperature reaction takes place. A temperature reaction was noted only in those animals in which a marked ophthalmic reaction was present. The local reaction was, therefore, sufficiently distinct to constitute a diagnosis, and the tedious task of taking the temperatures with two thermometers for greater accuracy is, from a practical point of view, superfluous. Only in special cases do we use the thermic reactions as an additional criterion of the mallein test. In field work, taking the temperature can be omitted. In case temperatures are taken (advisable for scientific investigation), they should be recorded at intervals of four hours. Schnürer recommends the taking of two temperatures, namely, one at the time of application of the mallein, and one at the first reading, about 12 hours after the instillation.

DURATION OF THE REACTION.

Usually the reaction remains visible for 12-36 hours after the application of the biologic product. There is no absolute rule. Many cases have been observed in which the local reaction was present only during six hours following its first occurrence, and, on the other hand, cases have been recorded in which the reaction remained visible for 72 hours. These observations are in accordance with those of de Blicke, Wladimiroff, and others. We found it advisable to read the reactions from the eighth hour after the application of the mallein, as then, frequently, the early reactions are distinctly visible. A second reading should be made on the 12th-16th hour, and usually at this time the height of the reaction is present. If possible, a reading after 24 hours should also be taken. Schnürer recommends for the testing of the reactions the 12th and the 24th hour; Miessner, the 14th-20th hour after the application.

In our experience, so-called "atypical" (Schnürer) reactions occur not uncommonly; viz., the conjunctival test appears and disappears suddenly, or the reaction is distinct only after 24 hours. Several horses which were kept in our stable showed such reactions. They were mostly in the stage of acute infection.

Every precaution should of course be taken to avoid the possi-

bility that the purulent discharge is not washed off by the stable man or caretaker; it will rarely happen that the animals, by mutual licking, will remove the discharge. From our experience, it is better not to advise the caretaker of the horses to be tested as to the interpretation of the alterations in the eyes, as otherwise the veterinarian is apt to be deceived when making retests in the same stable. In all such cases a repetition is highly recommended. Perhaps the other eye is used for the test.

RETEST.

Schnürer has found that a second application of mallein will, in nearly all doubtful cases, produce a marked positive reaction, or become entirely negative. From our observations with the ophthalmic test in tuberculosis, we were quite familiar with this fact, that in a sensitized eye the reaction is more legible than in an unsensitized one. This hypersensitiveness of the conjunctival membrane as a sequel of the first application of mallein is, however, noted only in glandered but not in healthy animals. Schnürer's observations on 3,000, and our records on about 400 healthy horses have shown that a conjunctival test does not sensitize as long as the animal is not infected with glanders. *Several horses were retested three and four times and still no reaction was recorded.*

Differing from the tuberculin test, the *retest can be applied 24 hours after the first application*, a very decided advantage under present conditions of city veterinary sanitary police, where in large stables a final decision is immediately desired. In most cases in which the first reaction was doubtful, a distinct or negative result was seen after the second test. Still, in two horses, which probably were in the stage of incubation, no reaction was obtained in either test. All observations stand in correlation with similar ones of Miessner, Schnürer, etc.

In many stables, all horses, including reacting (occult) ones, were retested a third time after 14 days, for the purpose of detecting such animals as were in the stage of incubation at the first and second tests, and of excluding the possibility of a simulated reaction in the reacting horses. Only when this third conjunctival test again gave negative results were the horses considered to be free from glanders.

On making this third test, and in one stable after a fourth and a fifth test, we observed that the degree of reaction became less and less distinct. Only a slight conjunctivitis was noted in animals which at first gave classic reactions. *For these reasons more than three retests should not be applied.* The mallein probably does not cause accumulation of leukocytes, and therefore no inflammatory process, on account of the adaption of the cells to the biochemic changes or on account of absence of complement concentration.

In this connection another important question should be considered, namely, after what incubation time does a recently infected animal give a positive conjunctival reaction? Müller, Gaehtgens, Aoki, and Miessner have shown that the conjunctival reaction is visible from the fifth to ninth days in artificially infected animals. In one case of Müller, Gaehtgens, and Aoki, the reaction was not apparent until the 18th day after the infection. We have not conducted special experiments, as the epidemiologic observations seemed to prove the findings of the said investigators. Fröhner concluded from the observations of Müller, Gaehtgens, and Aoki that the ophthalmic test should be preferred to the agglutination and complement fixation tests on account of the earlier positive results recorded—positive evidence when the serum test is still negative. This conclusion did not meet the approval of Miessner. My observations lead me to coincide with Fröhner. One particular case is recorded herewith.

A horse standing close to another which showed distinct glanders lesions on autopsy, developed a characteristic nasal discharge four days after it was removed from the infected stable (8-10 days after probable infection). The serum tests the first day were: agglutination 1:100; complement fixation, negative; the third day a marked ophthalmic reaction was noted; the fourth day, agglutination 1:500; complement fixation, negative. Based on the ophthalmic test, the horse was killed and glanders of the nasal septum, submaxillary lymph glands, and a few very small nodules in the lungs were demonstrated.

In a few other cases the ophthalmic test indicated distinctly the presence of a glanders infection at a time when the serum test did not indicate the slightest sign of a production of antibodies.

This occurred when, as was shown by autopsy, the glanders infection was slight and caused by *B. mallei* of low virulence (experiments). In these cases the production of agglutinins took place extremely slowly and over a long period of time, as in the cases recorded by Bonome. The conjunctival test, however, gave distinct reactions and allowed an early diagnosis.

In our opinion the conjunctival test is reliable for the determination of recently infected cases and certainly is, at this stage, frequently preferable to the serum tests (see p. 185).

CORRELATION OF THE CONJUNCTIVAL TEST TO THE SUBCUTANEOUS MALLEIN TEST.

The application of the subcutaneous mallein test is legally required in Pennsylvania and, therefore, the influence of this test on the conjunctival test was the subject of different observations. It is a well known fact that the subcutaneous application of mallein often suppresses somewhat the intensity of the local reactions, but this effect is, according to Foth, not marked nor of great practical importance. Many horses were tested by the subcutaneous method and afterward by the ophthalmic method and in no instance was a reaction noted when the horse was not infected with glanders. The ophthalmic test in most of the experimental cases followed the subcutaneous one in the next 24 hours. In several instances, 10-14 days elapsed before an ophthalmic test was made, and still no reaction was noted. In our experience the subcutaneous test does not influence a subsequent conjunctival test applied in the next 24 hours; a point which may be of great assistance to the practitioner. In an easy manner a doubtful temperature reaction can be checked by the ophthalmic test. As temporary febrile reactions are not uncommon in healthy draft horses, and as the failures of the subcutaneous mallein test in healthy animals, according to my statistical investigations, exceed 14 per cent, a simple test like the ophthalmic test will certainly be welcomed.

The subcutaneous mallein test, on the other hand, is not influenced by a preceding ophthalmic test. In case the conjunctival test was positive, a subcutaneous test applied during the following 3-14 days, causes a reappearance of the eye reaction often to a

much more distinct degree than at the time of the ophthalmic test. Animals in which such reactions occur are unquestionably affected with glanders; Schnürer says that the reappearance of past inflammatory symptoms of the conjunctiva during a subcutaneous malleinization is an absolutely conclusive proof of a glanders infection. Such conditions were frequently observed during our tests.

The simultaneous application of a subcutaneous and conjunctival test should be avoided, as in many animals during the febrile reaction a sudden fading away of the existing conjunctival reaction or a late appearance of it (after the fever has decreased) will be noted. The conditions are similar to those observed in tuberculosis (see Foth and others).

From our observations, we recommend the application of the conjunctival test first and, if necessary, the subcutaneous test in the second place and not in the reversed succession.

RESULTS OBTAINED AND GENERAL CONCLUSIONS.

In considering all the details mentioned, 210 horses were tested, from April until July. Since that date, about 400 additional horses were tested, checked by the serum tests and subsequent retests, but, as far as the autopsies and conjunctival tests are concerned, were not under my supervision. In the following table, therefore, are included only my own observations on 210 horses. The additional tests were as satisfactory as the experimental tests, and the deductions can, therefore, be applied to about 600 horses.

All our examinations as to the worth of the conjunctival reaction were conducted in conjunction with the laboratory tests, particularly the complement fixation and agglutination tests. The readings of the conjunctival reaction were made by the writer or by a trained assistant or agent of the Pennsylvania State Livestock Sanitary Board. The blood for the serum tests was collected before the mallein was instilled. The serum tests were all conducted by my first assistant under personal supervision in the laboratory, as usual and in the manner described in a recent publication. The autopsies of the animals found to be affected with glanders were conducted either in the postmortem room of the School of Veterinary Medicine, or in the field, in the presence of the writer. In

No.	CLINICAL SYMPTOMS	SERUM TEST I		MALLEIN SUBCUT. MAX. TEM.	OPH-THALMIC TEST I	SERUM TEST II		OPH-THALMIC TEST II	AUTOPSY
		Complement Fixation Test	Agglutination Test			Complement Fixation Test	Agglutination Test		
1		0.05	1:800		P ₃				N., L., and Lgl.
2			1:500		N				
3		0.1 influence by mallein	1:1000		N		1:600		
4		0.1	1:400		P ₃				N. and L.
5			1:800		N				
6	6-45 horses of one stable....								
7			1:200	103	N				
8			1:200	105	N				
9			1:200	103.3	N				
10			1:100	103.2	N				
11		0.02	1:100	104.2	N				
12			1:2000	103.1	P ₃				L. and Lgl.
13			1:500	105.4	N				
14		0.05	1:800	105.5	P ₃				L. and Lgl.
15		0.1	1:1000	104.4	P ₃				L. and Lgl.
16			1:800	105.5	N				
17		0.1	1:400	105	P ₁				L. and Lgl.
18			1:300	103.2	N				
19			1:400	103.3	N				
20			1:200	103.2	N				
21			1:100	104.2	N				
22			1:200	104.2	N				
23			1:200	103.5	N				
24			1:300	104	N				
25			1:400	103.1	N				
26			1:300	106	N				
27			1:400	102	N				
28			1:600	101.2	N				
29			1:300	102.2	N				
30			1:200	102	N				
31			1:200	102.1	N				
32			1:400	103.0	N				
33			1:400	101.2	N				
34			1:500	104.3	N				
35			1:400	103.2	N				
36			1:100	102	N				
37			1:200	104.2	N				
38			1:200	102	N				
39			1:600	103	N				
40			1:400	101.3	N				
41			1:100	102.3	N				
42			1:100	101	N				
43		0.02	1:200	100.2	N				
44			1:400	103	N				
45			1:300	102.2	N				
46	46-84 horses of one outbreak.		1:200	100.4	N				
47			1:200	102	N				
48			1:300	102	N				
49			1:500	103	N				
50			1:400	104	N				
51			1:300	103.3	N				
52			1:200	102.3	N				
53			1:100	104	N				
54			1:200	101	N				
55			1:400	104	N				
56			1:300	102	N				
57			1:200	102.2	N				
58			1:200	101	N				
59			1:100	103.3	N				
60			1:200	101.4	N				
61			1:400	101	N				
61]			1:300	102.2	N				

The horses of one and the same outbreak are indicated accordingly.

N. = nasal passages.

L. = lungs.

Sk. = skin.

Lgl. = lymph glands.

No.	CLINICAL SYMPTOMS	SERUM TEST I		MALLEIN SUBCUT. MAX. TEM.	OPH-THALMIC TEST I	SERUM TEST II		OPH-THALMIC TEST II	AUTOPSY
		Comple-ment Fixation Test	Aggluti-nation Test			Comple-ment Fixation Test	Aggluti-nation Test		
62			1:200	102	N				
63			1:200	104.2	N				
64			1:400	104	N				
65			1:200		N				
66			1:500		N				
67			1:200		N				
68		0.05	1:1500		P ₃				N. and L.
69	Farcy, nasal discharge	0.02	1:1000	pos.	P ₃				N. and L.
70		0.05	1:1500		P ₃				N. and L.
71			1:200	100	N				
72			1:400	100.2	N				
73			1:200	100.1	N				
74			1:100	100.2	N				
75		0.05	1:1000		P ₃				L. and Lgl.
76		0.05	1:2000		P ₃				L. and Lgl.
77		0.02	1:1000		P ₃				L. and Lgl.
78	Nasal discharge...	0.1	1:2000	105.4	P ₃				Acute infection N. and L.
79	Nasal discharge...	0.1	1:1000	103.4	P ₃				Acute infection N. and L.
80			1:300		N				
81			1:300		N				
82			1:500		N				
83	Farcy, nasal discharge	0.05	1:1000		P ₃				Culture obtained
84	Farcy, nasal discharge	0.05	1:2000		P ₂				Not made Culture ob- tained
85		0.1	1:1000	pos.	P ₄				N., L., and Lgl.
86			1:100	neg.	N				
87	Nasal discharge...		1:200		N				
88	Lesions on skin		1:400		N				
89			1:200		N				
90			1:400		N				
91			1:300		N				
92	Nasal discharge...	0.02	1:1000		P ₂				N. and L.
93	Nasal discharge...	0.02	1:800		P ₃				N. and L.
94	Nasal discharge...	0.05	1:600		P ₃				N. and L.
95			1:600		N				
96		0.05	1:1500		P ₃				N. and L.
97			1:800		Tem. 102.3				
98	Nasal discharge...	0.05	1:600		N				
99			1:200		N				
100			1:500		N		1:500	N	
101			1:1000		P ₃	0.1	1:1000	P ₃	N., L., and Lgl.
102			1:1000		P ₃	0.1	1:1000	P ₃	N., L., and Lgl.
103			1:1000		P ₃	0.1	1:1000	P ₃	N., L., and Lgl.
104			1:600		N				
105			1:400		N				
106			1:400		N				
107			1:600		N				
108	Nasal discharge...	0.05	1:1500		P ₃				N. and L.
109	109-145 horses of one outbreak				N		1:100	N	

No.	CLINICAL SYMPTOMS	SERUM TEST I		MALLEIN SUBCUT. MAX. TEM.	OPH-THALMIC TEST I	SERUM TEST II		OPH-THALMIC TEST II	AUTOPSY
		Comple-ment Fixation Test	Aggluti-nation Test			Comple-ment Fixation Test	Aggluti-nation Test		
110					P ₃	0.05	1:800	P ₁	L. and Lgl.
111							1:200	N	
112					N		1:100	N	
113				102.2	N	0.1 M	1:100	N	
114				(101.4)	N		1:200	N	
115				(101.4)	P ₂	0.05	1:200	P ₃	L. and Lgl.
				(104.8)				4 days on re-test	
116					N		1:300	N	
117							1:200	N	
118							1:100	N	
119							1:500	N	
120							1:200	N	
121				101.8	N	0.1 M	1:200	N	
122					N		1:200	N	
123				103.4	P ₁	0.05	1:300	P ₃	L. and Lgl.
124				103	P ₂	0.05	1:200	P ₂	L. and Lgl.
				(104.9)					
125				100.5	N	0.05 M	1:500	N	
126				100.4	N	0.1 M	1:100	N	
127					N		1:400	N	
128				104.4	P ₂	0.2 P	1:200	P ₁	L. and Lgl.
				(104.8)	(105.2)				
129				105.2	P ₃	0.2 P	1:200	P ₃	L. and Lgl.
				105.3	(104.4)				
130					N		1:100	N	
131					N		1:500	N	
132				103	P ₃	0.4 partial	1:1500	P ₁	L. and Lgl.
								(P ₃)	
133				105.4	P ₃	0.05	1:500	P ₂	L. and Lgl.
					(103.8)				
134				100			1:100	N	
135				101	P ₁	0.05	1:100		L. and Lgl.
					(101.4)				
136					N		1:100	N	
137					N		1:200	N	
138					N		1:400	N	
139					N		1:100		
140					N		1:200	N	
141					N		1:500	N	
142				104.4	P ₂	0.1	1:200	N	L. and Lgl.
				(103.6)	(103.5)				
143							1:300	N	
144							1:200	N	
145							1:200	N	
146	146-166 horses of one out-break		1:100	100.0					
147	Serum test only applied to remainder of animals								
148			1:100	102.2	N				
149			1:200	101	N				
150			1:200	103	N				
151				105.4	P ₂				L. and Lgl.
152				102.2	N				
153				103.4	N				
154				105.8	P ₃				L. and Lgl.
155				102.8	N				
156				100	N				
157				100	N				
158				102	N				
159				104.4	N				
160				103	P ₃				L. and Lgl., Sk.

No.	CLINICAL SYMPTOMS	SERUM TEST I		MALLEIN SUBCUT. MAX. TEM.	OPH-THALMIC TEST I	SERUM TEST II		OPH-THALMIC TEST II	AUTOPSY
		Comple-ment Fixation Test	Aggluti-nation Test			Comple-ment Fixation Test	Aggluti-nation Test		
161			1:200	105.4	N				
162			1:100	105.6	N				
163			1:200	103.2	N				
164			1:100	102	N				
165			1:200	101.2	N				
166			1:100	102.2	N				
167				104.6	P ₃				Sk., L., and Lgl.
168				104	P ₁	0.02	1:200		L. and Lgl.
169				105	P ₁	0.02	1:500		L. and Lgl.
170				104.4	P ₃				L. and Lgl.
171				104.2	P ₂				Sk., L., and Lgl.
172		0.05	1:1500		P ₂				Sk., L., and Lgl.
173			1:200		N				
174			1:200		N				
175			1:500		N				
176			1:200		N				
177			1:100		N				
178			1:200		N				
179			1:200		P ₂	0.05	1:1000	P ₂	Isol.
180			1:100		N		1:100		
181		0.02	1:2000		P ₂				Sk. and L.
182		0.01	1:4000		P ₁				Sk. and L.
183			1:400	101.5	N				
184		0.3	1:1000	104.5	P ₃				Sk. and L.
185			1:100	103.6	N				
186			1:200		N				Sk., L., and Lgl.
187		0.01	1:4000		P ₃ (₄₈ after applica- tion)				Sk. and L.
188		0.02	1:1000		P ₃				Sk., L., and Lgl.
189	Nasal dis-charge...	0.02	1:1500		P ₃				L. and Lgl. and N.
190	Nasal dis-charge...	0.01	1:4000		P ₃				L. and Lgl. N.
191		0.2	1:1000		P ₁				L. and Lgl.
192	Nasal dis-charge...	0.1	1:1500		P ₃				L. and N.
193			1:400		N				
194	Discharge from both nostrils ..								
195	195-208 horses of one out-break....	0.1	1:800		P ₄				N. and L.
196				100.2	N		1:200		
197				103.1	N		1:500		
198				100	N		1:200		
199				100.2	N		1:500		
200				100.2	N		1:500		
201				103.1	N		1:300		
202				100.3	N		1:400		
203				101.2	N		1:200		
204				100.2	N		1:300		
205				100.4	N		1:600		
206				100	N		1:200		
207				100.2	N		1:500		N., Sk., L., and Lgl.
208				104.8	P ₄	(0.02)	1:1000		
209				103	N	0.05	1:800		L. and Lgl.
210					N		1:100		
210					N		1:200		

a few instances where clinical cases were diagnosed, a detailed autopsy was omitted when the *B. mallei* had been isolated from the organs or secretions submitted for examination. As quite a few veterinarians still consider the Strauss reaction to be the chief diagnostic method for glanders, material is frequently submitted for laboratory diagnosis without our request, and has been successfully used for the inoculations mentioned before a test was applied. In rare cases the animals were tested only by the ophthalmic method and were killed before the serum tests were concluded. In such cases the autopsies were made with particular care and, if necessary, animals were inoculated. The material was not especially selected but came from different outbreaks of glanders which came to the notice of the Pennsylvania State Livestock Sanitary Board. In most instances the animals tested did not show any clinical symptoms (occult cases of glanders) and thus gave the conjunctival test a more severe trial.

The results are given in the table, which shows that of 210 horses, 58 were found by means of the complement fixation test to be suffering from glanders. The interpretation of this test is based on the principle as outlined by Miessner and others and shown in my publication on this subject. Statistical investigations (see publications from 1909-12) show that 99.6 per cent correct results in glanders and 99.75 per cent in healthy horses are obtained with the complement fixation test. Of the 58 glanders cases, only 56 reacted positively to the conjunctival test, while two horses which proved at postmortem to be affected with glanders did not give any reaction whatever. On account of the positive serum reactions, both animals were condemned and, therefore, a third retest at 14 days' interval could not be carried out. The two animals, Nos. 206 and 208, were, according to the history and the result of the serum tests, in the stage of incubation and would probably have shown a positive reaction on a third retest. In the experimental horse No. 28 conditions observed by Müller, Gaetgens, and Aoki were therefore existing, namely, *in quite recent infections the conjunctival tests may be negative and occur only several days after the appearance of the antibodies in the serum of the patient.* Whether or not this is an exception has to be determined by further

observations. We found lately that these conditions are rare (see also p. 178).

The retest, 14 days after the first test, gave, with a few exceptions, distinct results. Miessner concluded from his observations that in many cases, probably on account of a certain adaptation (also in glanders horses), only a slight reaction will be noted. In his opinion, only the complement fixation test should be used for a retest in a stable. In our opinion, the second serum test can very readily be combined with a third ophthalmic test.

As previously explained, the subcutaneous mallein test (legal requirement) was applied several times at 14-day intervals. The horses (for example, No. 115 and No. 135) became gradually used to the mallein and did not show any febrile reactions, and yet the ophthalmic mallein test was, in all instances, positive. This fact is of great importance, particularly when the serum tests, on account of a previous mallein application, are misleading. In horses that are maliciously injected with mallein to veil the results of a subsequent test by a state official, the conjunctival test will be of great assistance in disclosing the true condition. So far as we know, the use of antipyretics to falsify the mallein test is not commonly practiced, and the results of the ophthalmic test under these conditions have to be determined. Experiments for this purpose are in progress. In what manner the mallein vaccine used in New York City influences the conjunctival test has also to be determined. Practical experience has shown that the serum tests are misleading (over a long period agglutinins and complement-fixing antibodies are present).

In other diseases, particularly sporotrichosis, with all its clinical similarities to glanders, a positive reaction to the ophthalmic test was never obtained.

To give a few examples of the readings of the reactions, two characteristic cases are selected. The limited space at our disposal will not permit the giving of all the readings in detail as has been done in other publications (Schnürer, Fröhner, Reinhardt, Miessner, and others).

No. 1: Horse, bay gelding; temperature 100.8° F. at 9 P.M. Showed 10-5-12, 10 hours after the application of the ophthalmic mallein, severe lacrimation, photophobia, profuse purulent discharge, temperature 104.8.

Serum test.—Complement fixation, 0.05 binding value, agglutination 1:200. The animal was retested 29-7-12 and showed for three days a severe purulent discharge, lacrimation, and photophobia. Complement fixation 0.05, agglutination 1:400. This animal was killed and the autopsy showed four old glanders foci in lungs and two large cheesy glanders nodules in the bronchial lymph glands.

No. 2: Horse, bay, 10 years old, weight 1,300 lbs., condition very good; sub-maxillary lymph glands, slightly hardened; few small nodules 10 inches above the haunch. Ophthalmic mallein applied 8 P.M. First observation 8 A.M., 8-10-12, eye showed slight lacrimation and photophobia. At the inner canthus of the eye was a very small amount of whitish slimy material. Second test applied 8-10-12, 10 P.M. First observations 8 A.M., 8-11-12. Excessive lacrimation, pronounced photophobia, edematous swelling of the lids, and considerable amount of whitish yellow pus-like discharge at the inner canthus. Complement fixation 0.02; agglutination 1:1500.

Postmortem examination.—Glanders of the lungs, the bronchial and submaxillary lymph glands and integumentum.

In the table is also shown that not one of the healthy horses (152) gave a positive ophthalmic reaction. That these horses were really not affected with glanders was proven primarily by successive serum tests and a careful observation and control during the last five months of all the stables in which the test was applied. No further cases of glanders have developed and the disease can, therefore, be considered as having been eradicated. In several of the stables, only recently serum tests were conducted and not one of the animals has reacted. The results obtained in the healthy horses correspond with those mentioned by Reinhardt, Wladimiroff, Klimmer,¹ Dedjulin,² Miessner, and others; 100 per cent correct results were obtained by these investigators. Schnürer reports 22.3 per cent failures in testing 5,450 animals. These results are based upon reports which were submitted by veterinarians, many of whom had not the training necessary to interpret the reactions. Such mistakes will undoubtedly be eliminated in the future when the method has become perfectly familiar to the profession. In apparently healthy horses a reaction to the conjunctival test followed by failure to demonstrate anatomical lesions certainly should not be considered as proof of the inefficiency of this test. Everyone familiar with the results of the tuberculin test will be prepared to admit that also in glanders similar conditions occasionally prevail, and that only a most care-

¹ *Handbuch der Serum-Therapie und Serum-Diagnostik in der Veterinär Medizin*, 1911, p. 321.

² *Ztschr. f. Infektionskrankh. usw. d. Haustiere*, 1912, 15, p. 365.

ful autopsy will reveal minute anatomical lesions. As autopsies are not very agreeable to the veterinarian under usual conditions, the number of failures reported out of a large number of animals tested will naturally be greater than are recorded in the few tests in this paper.

All in all, the conjunctival method certainly cannot be blamed for these failures, and, compared with the subcutaneous mallein test, the results obtained are remarkably accurate. In addition, the simple manner of application, the relief from time-absorbing taking of temperatures with all its disadvantages, will certainly place this test in the first rank of the diagnostic methods for glanders.

THE RESULTS COMPARED WITH THE COMPLEMENT FIXATION AND AGGLUTINATION TESTS.

In the interest of a perfect sanitary control, a centralized record system of all glanders cases must be maintained. To enforce such a legalized system it is necessary that the diagnosis of glanders be established independently of the practitioner, especially as the state is giving compensation for the destruction of the animals. The checking of the field tests for glanders and the confirmation of the diagnosis is most efficiently done in the state of Pennsylvania by the serum tests.

In the table, the results are shown and compared with similar observations of Dedjulin and Miessner. We have noted the very satisfactory and remarkably accurate results with the complement fixation test. In a recent publication special emphasis has been paid to this fact.¹

As mentioned before, in the state of Pennsylvania the use of the subcutaneous mallein test is still required. It was therefore not surprising to find that several sera in our tests gave positive reactions in the complement fixation test when there was no sign whatever of glanders, and when the retest proved that such animals were not suffering from the disease. We had 3.2 per cent failures in the healthy horses and attribute this fact to the production of immune bodies by previous injections of mallein. *We earnestly*

¹ K. F. Meyer, *Proc. of the Amer. Vet. Med. Assn.*, 1912.

request, therefore, that the proper authorities arrange that when a serum test is to be applied, no subcutaneous mallein test be made; a request which is reasonable on account of the facts explained, and which has been recognized as proper procedure in Prussia (for the last four years). The veterinary sanitary laws of Prussia forbid the use of mallein (subcutaneously) on account of its effect on the interpretation of the serum tests.

The agglutination test, in considering 1:800 a reaction, showed rather unfavorable results, as by it alone only 74.5 per cent of the actual cases would have been detected. By it 2.6 per cent of the healthy horses gave a positive reaction, and might have been considered as suffering from glanders. The agglutination test alone would therefore have been very unreliable. This observation has been critically discussed in my last publication. It is impossible to mark a certain *limes titre* as an indication of a positive reaction. Only reactions of 1:2000 and above this titre can be considered as conclusive. Since the introduction of the complement fixation test, we use the agglutination test as a control only for the period of incubation, and never has a diagnosis been made based solely on the result of the agglutination test. We will retain the agglutination test as a laboratory method and not follow the proposal of Miessner to replace the agglutination test by the conjunctival test. These conclusions stand in correlation with those of Schnürer, Frohner, Reinhardt, and others.

A few observations were made as to the effect of the ophthalmic mallein on the serum reactions. In no instance was an increase of the agglutinins and complement-fixing antibodies noted. The observations of Miessner made on glandered horses are not conclusive, as animals were selected in which the increase of the antibodies as a natural sequel of the infection was to be expected.

CONCLUSIONS AND RECOMMENDATIONS.

In considering these investigations, the following conclusions can be drawn:

1. The conjunctival test for glanders is very reliable. It can, in a short time, without large expense, be applied by every prac-

ticing veterinarian and will permit the untrained to make a diagnosis of glanders with the greatest possible accuracy.

2. The serum tests are necessary to centralize the control of infectious diseases in a reliable state institution and to support the diagnosis in case compensation is sought by the owner of the animal. Only the complement fixation test can be used independently for the diagnosis of glanders.

In fulfilling these requirements, the following plan is recommended: The practicing veterinarian obtains from the state laboratory the mallein preparation, eye-droppers, test tubes, and needles for the collection of blood. On a special report blank the number, name, and position of the horses in the stable are noted. Then blood is collected (carefully considering the precautions mentioned in a special circular letter), marked in correspondence with the numbers on the report, and immediately forwarded to the laboratory. Simultaneously, a conjunctival test is made by dropping into the conjunctival sac (on the mucous membrane of the lower lid) two drops of a 5 per cent (1 per cent) solution of "mallein siccum." The solution must be made shortly before use. About 10-24 hours afterward, two examinations of the instilled eye are to be made. The degree of reaction is best marked on the report as follows:

Absence of reaction.....	N
A slimy discharge.....	D
A purulent discharge.....	P1
A purulent discharge and swelling of the eyelids.....	P2
An abundant purulent discharge with photophobia, lacrimation, etc.....	P3-P4

In cases where a doubtful reaction is obtained, a retest on the same eye with the same amount of mallein is made after the reading (20-24 hours after the first application). After 8-20 hours the eye is examined again and the result noted. All reacting animals are to be carefully isolated. In considering the result of the complement fixation and agglutination tests, the animals affected with glanders are proposed for destruction and if possible disposed of.

Fourteen days after the isolation, or better, the destruction, of the glandered horses, a third retest of the remaining animals, including, perhaps, the doubtful reactors, with ophthalmic mallein

and the serum tests is made. Should further cases of glanders be detected by the third, a *third repetition of the serum test alone* should be ordered. The subcutaneous mallein method should be omitted, or in case it has been applied, proper information should be sent to the one conducting the tests.

This plan for the diagnosing of glanders has been used with success during the last few months in the state of Pennsylvania.